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Electrochemical amperometric immunoassay for carcinoembryonic antigen based on bi-layer nano-Au and nickel hexacyanoferrates nanoparticles modified glassy carbon electrode

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ABSTRACT

This study demonstrates a new approach towards development of novel immunosensor based on gold nanoparticles (nano-Au) and nickel hexacyanoferrates nanoparticles (NiHCFNPs) for determination of carcinoembryonic antigen (CEA) in clinical immunoassay. The fabrication steps of the immunosensor as follows: firstly, nano-Au was immobilized on the surface of bare glassy carbon electrode (GCE) by using a simple method – electrochemical reduction of $HAuCl_4$ solution; secondly, NiHCFNPs as an electroactive substance was immobilized on the layer of gold nanoparticles. Microstructure and surface morphology of NiHCFNPs have been characterized by transmission electron microscopy (TEM) and scanning electron microscopy (SEM); thirdly, nano-Au was again immobilized on the surface of NiHCFNPs, which can offer a favorable microenvironment and biocompatibility to immobilize anti-CEA. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were applied to characterize the electrochemical properties of modified process. Effect of deposition time of nano-Au, pH of working buffer, incubation temperature and time were studied in detail for optimization of analytical performance. Under optimal conditions, the peak current of CV of the immunosensor decreased linearly with increasing CEA concentration in two ranges from 0.5 to 10.0 ng mL^{-1} and from 10.0 to 160.0 ng mL^{-1}, with a detection limit 0.1 ng mL^{-1} at three times background noise. The proposed immunosensor show good repeatability and reproducibility, acceptable accuracy, high sensitivity and would be valuable for diagnosis and monitoring of carcinoma.

1. Introduction

In the tumor process, increased level of tumor markers in human serum, are associated with certain tumor. Therefore, determination of tumor markers, potential prognostic factors for tumors, plays an important role in clinical research and diagnosis [1,2]. Carcinoembryonic antigen (CEA), is typically associated with certain tumors and the developing fetus [3,4] and is widely used as clinical tumor marker for some familiar cancers [5–8]. The normal range for CEA in an adult non-smoker is <2.5 ng mL^{-1} and for a smoker <5.0 ng mL^{-1}. A rising CEA level indicates progression or recurrence of the cancer [9,10]. Thus, the detection of CEA levels in human serum is necessary in clinical assay.

The technique based on high specific molecular recognition of antigen by antibody and used for quantitative determination of tumor markers is usually immunoassay. Many kinds of immunoassay methods [11–17], have been applied for the detection of CEA. However, methods described above have always the disadvantages, such as time consuming, requiring highly qualified personnel, sophisticated instrumentation, poor precision, difficult to realize automation [18,19]. Electrochemical immunoassay combining the features of fast analysis, sensitive and precise measurement, simple pretreatment, inexpensive and miniaturizable instrumentation, has drown more attention in a wide range of uses [20]. Electrochemical amperometric immunoassay with amperometric transducer is receiving intense attention because it can achieve a relatively low detection limit and high sensitivity. The amperometric immunoassay also plays an increasing role in immunosensors. Thus, looking for a novel immobilization method for amperometric immunosensor with great improvement in sensitivity, selectivity, and response time is of considerable interest.

Advanced material based on inorganic and organic nanoparticles is nowadays one of the key research fields of today material science. Among these nanomaterials, noble metal nanoparticles have been used to fabricate biosensor owing to their excellent properties to immobilize biomolecules (such as enzyme, antibody, antigen, DNA, RNA) [21–24]. The nano-Au has drawn much attention in constructing electrical and optical sensors due to their small size and correspondingly unique optical, electronic,

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and catalysis properties. The nano-Au not only can offer a microenvironment similar to nature and retain the bioactivity of the immobilized biomolecules, but also can provide a high surface to volume ratio and enhance the electron transfer kinetics by giving more freedom energy to the immobilized biomolecular in orientation which makes active sites closer to conducting electrode and permit the biomolecules to orient in conformation more favorable for direct electron transfer [25,26]. In our present work, a nano-Au layer on the electrode was prepared by using a simple method – electrochemical reduction of $HAuCl_4$ solution that agrees with literatures [27–29]. The nano-Au film formed by this method can be achieved in a relatively short time and can provide a stable and rough surface that is more favorable to immobilize biomolecules.

Metal hexacyanoferrate complexes (MHCFs) modified electrodes have attracted considerable interest due to their potential application in many fields [30,31]. Among PB analogues, nickel hexacyanoferrate exhibits attractive redox properties in term of charge compensation [32]. In addition, it has been to the electrocatalysis and determination of organic and inorganic species [33]. So far, little attention has paid to use nickel hexacyanoferrate nanoparticles (NiHCFNPs) to construct biosensors. NiHCFNPs is of lager surface area, a high surface to volume ratio, and high affinity to nano-Au due to existing strong interaction between $-NH_2$, –SH, –CN, and nano-Au [34–37]. It will be an important improvement to modify NiHCFNPs onto nano-Au layer for biosensing application. In the present work, NiHCFNPs was synthesized by simply mixing nickel ions and hexacyanoferrate ($K_3Fe(CN)_6$) at room temperature. After that, NiHCFNPs as a good electroactive substance at the same time was immobilized on the electrode, which greatly simplifies the immunoassay system. However, many amperometric immunoassay techniques need an electroactive substance to analytical system, which lead to more complex immunoassay system and increased analytical time and expense [38,39].

Recently, we also developed some electrochemical immunosensors based on nanomaterials with favorable biocompatibility for the determination of CEA [40–42]. Sensitivities of the immunosensors need to be improved. In the present investigation, we simultaneously took advantages of two kinds of nanomaterial (nano-Au and NiHCFNPs) with good properties, tried to develop a simple and sensitive immobilization strategy for constructing a compatible amperometric immunoassay. At first, nano-Au layer with favorable biocompatibility and large surface area was formed by simple electrochemical reduction method. Secondly, NiHCFNPs as a good electroactive substance was self-assembled on nano-Au layer by strong interaction between CN^- (NiHCFNPs) and nano-Au [34–37]. Thus, the second nano-Au layer was again self-assembled onto NiHCFNPs by simply electrochemical reduction, which offers a biocompatible interface to adsorb anti-CEA by chemical adsorption between nano-Au and $-NH_2$ of anti-CEA. In addition, nano-Au layer formed by this method can offer a stable, rough and compact surface, which can immobilize more amount antibody. There are two novel strategies in designing the immunosensor as follows: First, promising polymeric inorganic compound NiHCFNPs was not only an excellent electroactive substance, but also could provide a favorable interface for nano-Au immobilization, which greatly improve performance of immunosensor. The nano-Au and NiHCFNPs both with excellent properties were simultaneously used to design an accuracy, sensitivity and stability immunosensor. Second, the preparation of immunosensor is simple, easy controlled and less time consuming. The performance and factors influencing the immunosensor's performance were also investigated in detail. The proposed immunosensor was applied to determine CEA in human serum samples with satisfactory results.

2. Experimental

2.1. Reagents and materials

CEA and anti-CEA (Biocell Company, Zhengzhou, China), Bovine serum albumin (BSA, 96–99%), gold chloride ($HAuCl_4$) and sodium citrate (Sigma, St. Louis, MO, USA), $K_3Fe(CN)_6$ and $NiCl_2{\cdot}6H_2O$ (Chemical Reagent Co, Sichuan, China). All other chemicals and solvents (analytical grade, regular sources), Bi-distilled water was employed throughout this study. Phosphate buffered solutions (PBS) at various pH were prepared using 0.02 M Na_2HPO_4 and 0.02 M KH_2PO_4 stock solution. The supporting electrolyte was 0.1 M KCl. The CEA was stored in the frozen state, and its standard solutions were prepared freshly with bi-distilled water when in use.

2.2. Apparatus

CHI 610A electrochemistry workstation (Shanghai CH Instruments, China), Model IM6e (ZAHNER Elektrick, Germany), pH meter and digital ion analyzer (Model PHS-3C, DaPu Instruments, Shanghai, China), transmission electron microscopy (TEM) (TECNAI 10, PHILIPS, Holand), S-3400 scanning electron microscope (SEM) (Hitashi High Technologies Corporation, Japan).

2.3. Preparation of nickel hexacyanoferrates nanoparticles (NiHCFNPs)

The synthesis of NiHCFNPs was according to the literature [43] with a little modification: 70 mL of 0.01 M $NiCl_2$ aqueous solution was drop by drop added to 70 mL of 0.05 M $K_3Fe(CN)_6$ aqueous solution containing 0.05 M KCl under stirring. After finished addition, the mixture solution was vigorously agitated for 5 min. Following that, the mixture solution was immediately centrifuged, and washed with bi-distilled water for several times. Then dried NiHCFNPs overnight in a vacuum at room temperature and finally gave a powered substance.

2.4. Preparation of the immunosensor

The glassy carbon electrode (GCE) (Φ = 4 mm) was first polished to a mirror finish respectively with 1.0, 0.3 μm alumina slurry, followed by rinsing thoroughly with bi-distilled water after each polishing step. After that, the electrodes were successively sonicated in 1:1 nitric acid, ethanol, bi-distilled water. Following dried in air.

The electrode was modified immediately after the cleaning steps. The preparation of nano-Au layer was constructed by immersed the cleaning electrode in $HAuCl_4$ aqueous solution (2 mg mL^{-1}) and applied constant potential −0.2 V for 60 s. It was rinsed with a copious amount of water and a yellow nano-Au layer can be seen.

Before the step of modification, 0.05 M NiHCFNPs aqueous solution need be prepared firstly. When NiHCFNPs was dispersed in bi-distilled water, its aqueous solution was very stable and no precipitate was observed after 2 months when stored at 4 °C. A 10 μL NiHCFNPs aqueous solution (0.05 M) was dropped onto the surface of nano-Au layer formed right now. Immediately transfer the electrode to refrigerator and keep at 4 °C for about 2 h for NiHCFNPs film dry. When NiHCFNPs film dried, the electrode was washed thoroughly with bi-distilled water.

The NiHCFNPs/nano-Au modified electrode was soaked in 3 mL $HAuCl_4$ aqueous solution (2 mg mL^{-1}) and applied constant potential −0.2 V for 30 s. Following that, the modified electrode washed with bi-distilled water. Subsequently, the nano-Au/NiHCFNPs/nano-Au modified electrode was immersed in anti-CEA solution at 4 °C for about 12 h. Finally, the proposed electrode was incubated